Comparison of the sensitivity of early life stages of freshwater mussels to current-use pesticides

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Outline

- Background & justification
- Goal & objectives
- Technical grade tests
- Formulation tests
- Conclusions
- Planned studies

Family Unionidae – *The Freshwater Mussels*

- **Most imperiled faunal group in world**
  - **~300 sp. in N.A.**
  - **~67% endangered, threatened, of special concern, or extinct**
- **Decline since 1800s**
  - **hastened past 50 yrs**
  - **numerous factors**
  - **biological & physical**

Pesticides

- One of many potential factors
- Current-use pesticides not persistent
- Application coincides with reproduction
- Early life stages most sensitive to other contaminants
- Paucity of information

Goal

Generate toxicological information on the hazards of a suite of current-use pesticides to early life stages of freshwater mussels

Objectives

- Determine the acute & chronic toxicity of technical grade pesticides to glochidia and juveniles of selected mussel species
- Determine acute toxicity of a reference toxicant (NaCl) to early life stages of mussels
- Assess the acute & chronic toxicity of pesticide formulations to of glochidia and juveniles of a selected species

Phase I

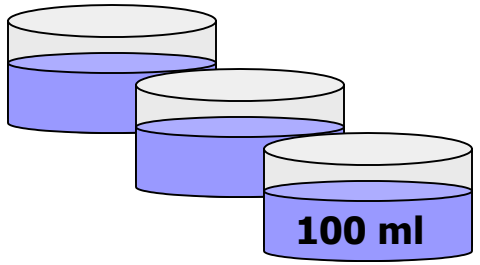
Technical grade pesticides

Test Conditions

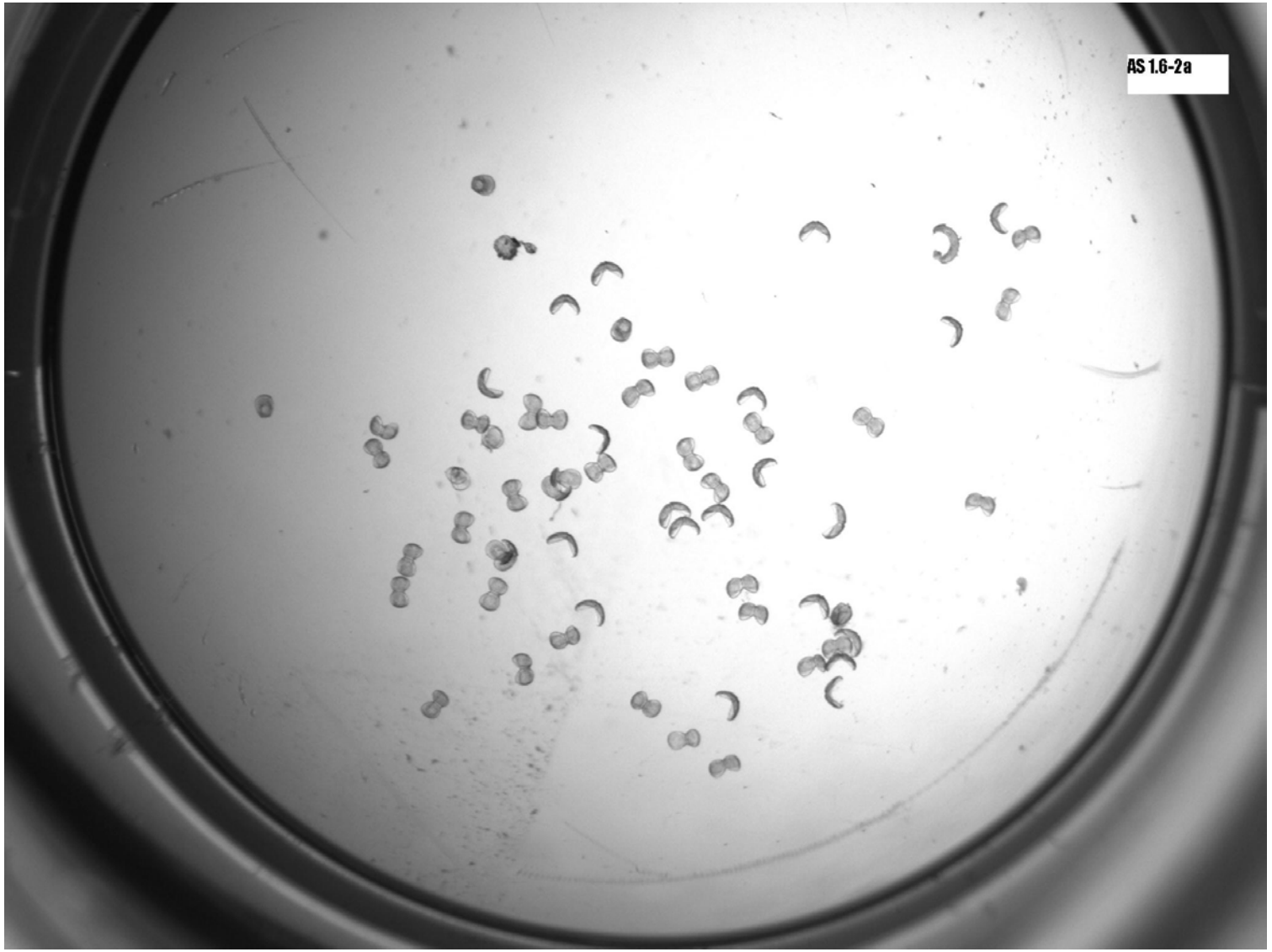
- ASTM standard for mussel tox testing (2005)
- ASTM hard water
  - Hardness ~ 180 mg $CaCO_3$/L
  - Alkalinity ~ 120 mg $CaCO_3$/L
  - pH 8.3 – 8.6
  - Temp. 20 $\pm$ 1^{o}C
  - D.O. > 80% sat.
  - Conductivity ~ 600 μS/cm
- Technical grade atrazine, fipronil, pendimethalin, permethrin

Technical grade & glochidia

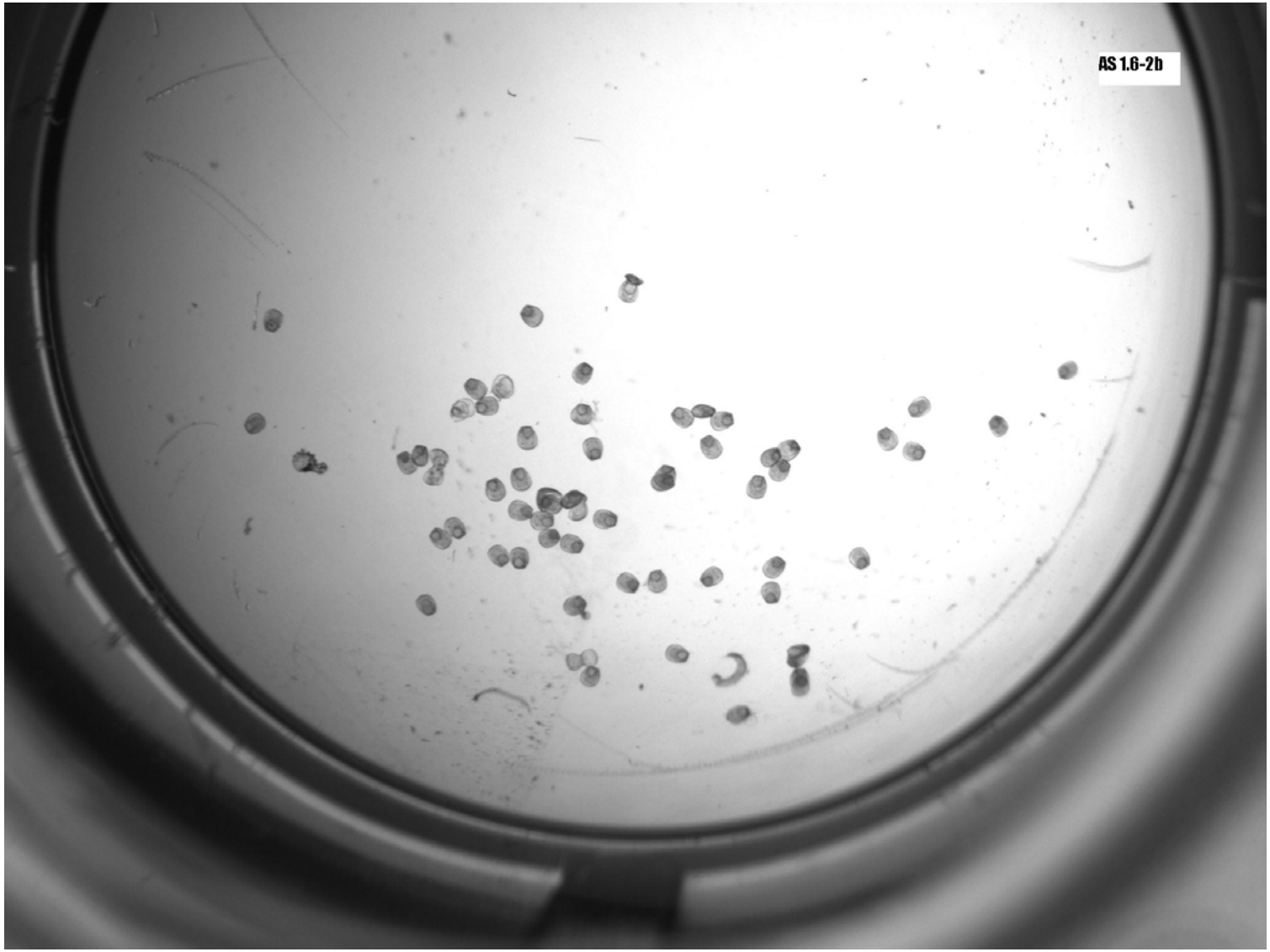
- 6 species
  - *Elliptio complanata, Lampsilis fasciola, L. siliquoidea, Utterbackia imbecillis, Villosa delumbis, V. constricta*
- 7 treatments (to solubility)
- 100–150 glochidia/replicate
- 48-h static exposure
- Verify exposure, water chemistry (t0, 48)
- Endpoint: survival (24, 48 h)
  - subsample of 50 glochidia
  - response to saturated NaCl
- LC50s: Spearman-Karber Method



Photodocumentation: Pre-NaCl

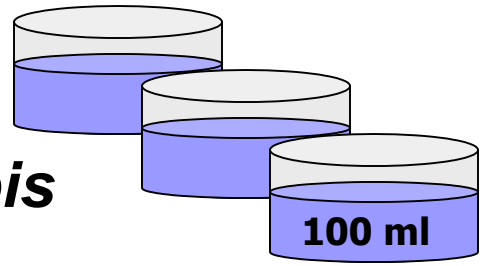


Post-NaCl



Technical grade & juveniles

- Same pesticides, concentrations
- 5 species
  - *Lampsilis fasciola, L. siliquoidea, L. abrupta, Ligumia recta, Villosa delumbis*



- < 5 d, 2 mo. post-release
- 7 juveniles/replicate
- 96-h static renewal (48 h)
- Validate exposure (t 0, 96)
- Endpoint: survival (48, 96 h)
  - foot movement (5 min)
- Spearman-Karber Method

Results (Phase I)

Results: Glochidia

- 32 Acute tests
- Exposure validation (% of target)

	t 0	t 48
Atrazine	74 – 91%	56 – 88%
Fipronil	40 – 57%	23 – 57%
Pendimethalin	113 – 120%	83 – 113%
Permethrin	50 – 80%	25 – 51%

- Low or no mortality with all species

  LC50s > highest test concentration (solubility)

Results: Juveniles

- 18 Acute tests
- Exposure validation (% of target)

	t 0	t 96
Atrazine	83 – 103%	83 – 95%
Fipronil	43 – 68%	44 – 53%
Pendimethalin	83 – 132%	73 – 93%
Permethrin	75 – 102%	20 – 50%

- Low or no mortality with all species

  LC50s > highest test concentration (solubility)

NaCl (reference toxicant) LC50s

Glochidia

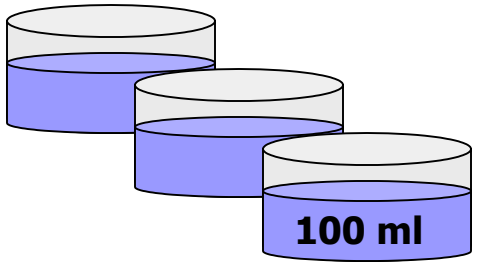
0.56 g/L (*L.s.*) – 3.63 g/L (*V.d.*)

Juveniles

3.98 g/L (*L.f.*) – 6.31 g/L (*L.s.*)

Chronic toxicity: juveniles

- *L. siliquoidea* (4 month old)
- Atrazine (tech. grade)
- 21-d static renewal (48-72 h)
- 7 juveniles/rep
- Fed live *Neochloris* daily
- d 7, 14, 21
  - exposure validation
  - survival (movement)
  - length measurement
    - image analysis



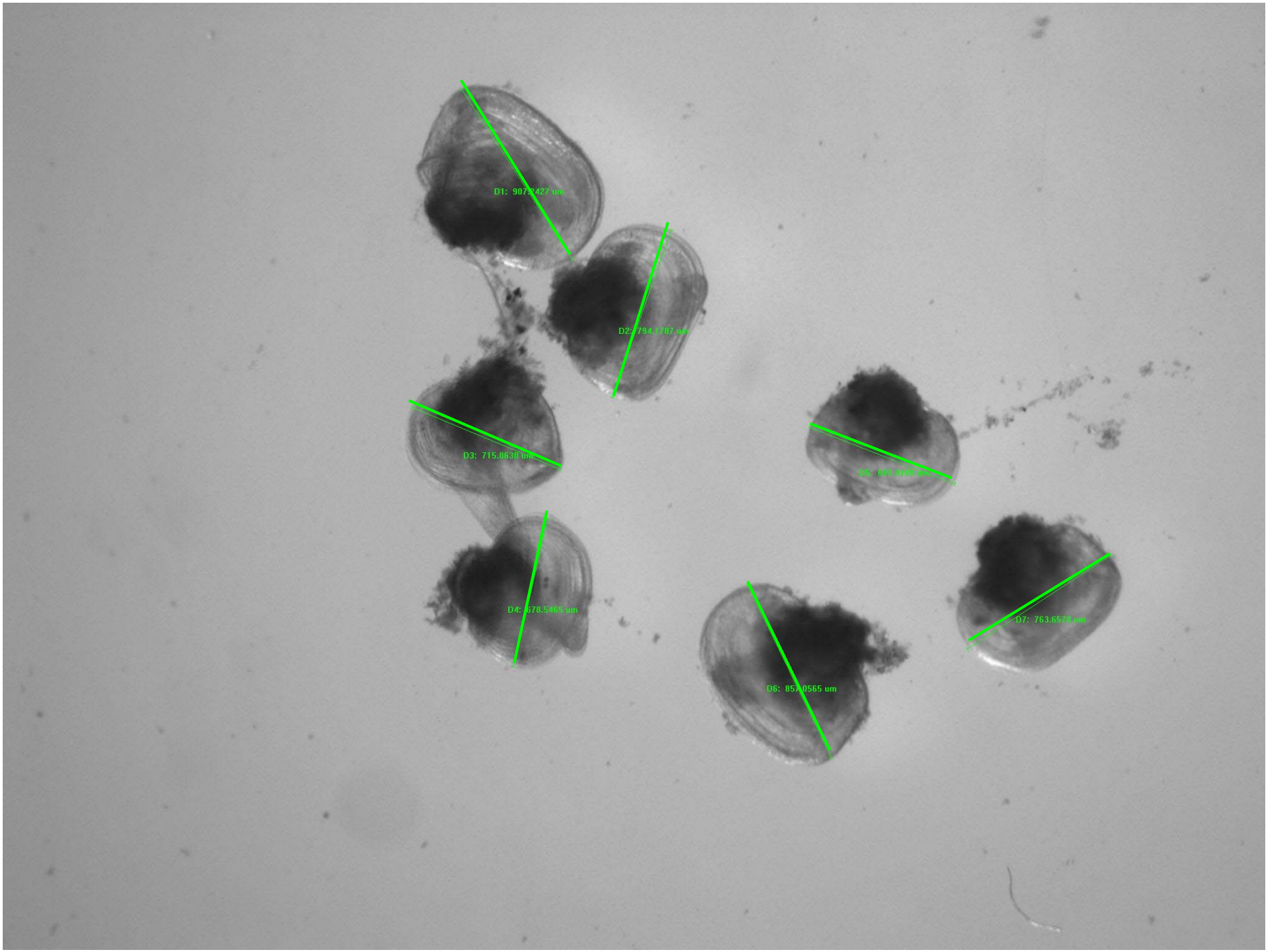
Results:
Atrazine - chronic test

- **Exposure validation**

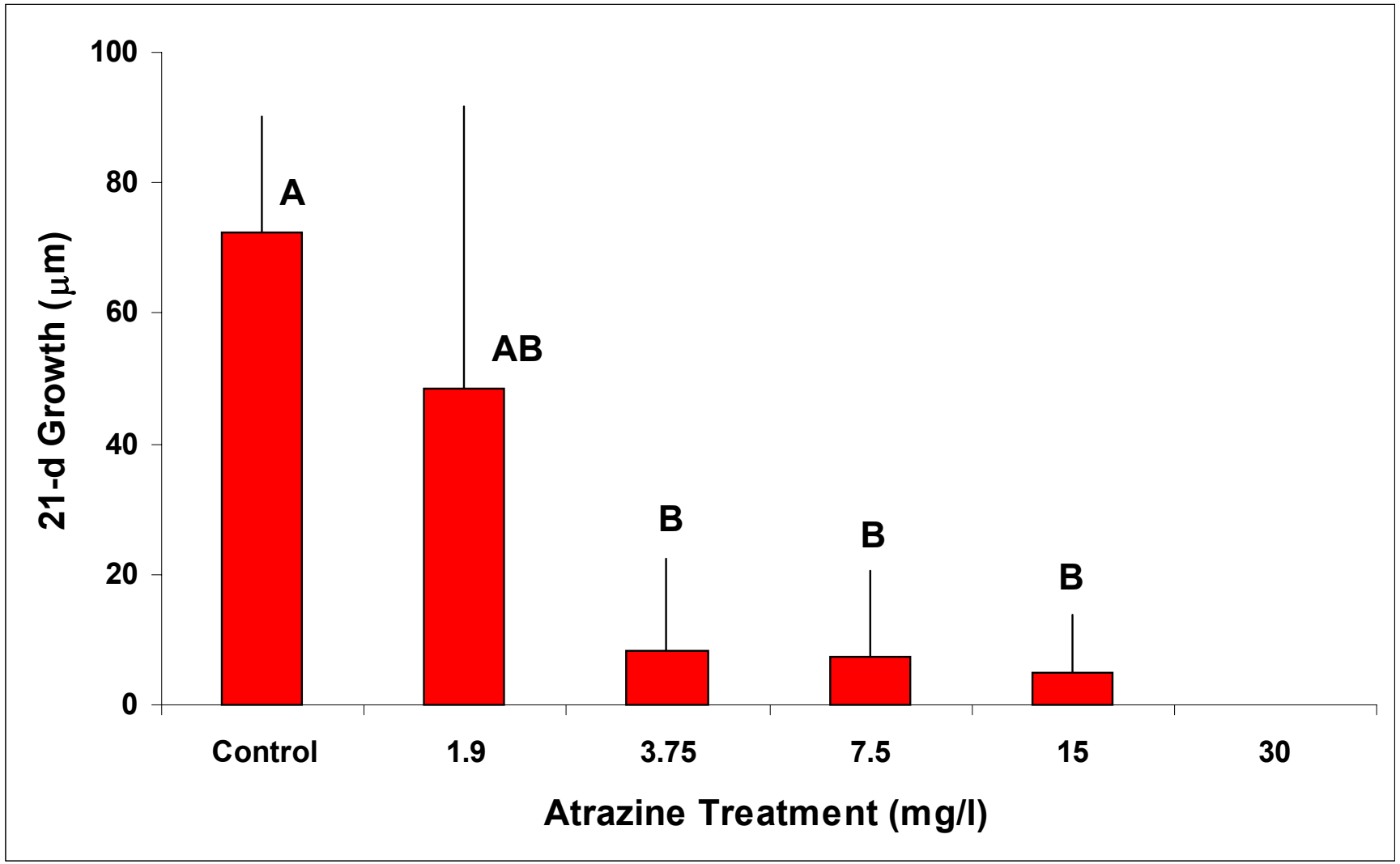
  83 – 133% of target

- **LC50s & 95% C.I. (mg/L)**

d 7	d 14	d 21
> 30	15.8	4.3
	(12.0 - 19.5)	(2.8 - 5.8)


D1: 907.3427 um
D2: 794.1787 um
D3: 715.8630 um
D4: 678.5465 um
D6: 854.0565 um
D7: 763.6576 um

Growth


100
80
60
40
20
0
21-d Growth (μm)
A
AB
B
B
B
Control
1.9
3.75
7.5
15
30
Atrazine Treatment (mg/l)

Results summary: Phase I

- Technical grade atrazine, fipronil, pendimethalin, permethrin not acutely toxic to glochidia or juveniles
- NaCl more toxic to glochidia than juveniles
  - suitable reference toxicant
- Juvenile L.s. 21-d atrazine LC50: 4.3 mg/L
  - environmental relevance?
- Chronic effects of atrazine on juvenile L.s. growth

Phase II

Formulation pesticides

Test Conditions

- ASTM standard for mussel tox testing (2005)
- ASTM hard water
- Acute and chronic
- *L. siliquoidea* only

Pesticide formulations

Technical grade	Formulation
atrazine	AAtrex® 4L
chlorpyrifos	Lorsban® 4E
glyphosate	Roundup Ultramax®
permethrin	Mosquito-B-Gone®

Results (Phase II)

LC50s & 95% C.I. (mg/L)

Pesticide	Glochidia		Juveniles	
	24 h	48 h	48 h	96 h
glyphosate	> 200	> 200	> 200	> 200
Roundup®	4.21 (2.83, 6.28)	4.00 (2.83, 5.65)	7.96 (5.47, 11.58)	7.96 (5.47, 11.58)
atrazine	> 30	> 30	> 30	> 30
AAtrex®	> 30	> 30	> 30	> 30

LC50s & 95% C.I. (mg/L)

Pesticide	Glochidia		Juveniles	
	24 h	48 h	48 h	96 h
chlorpyrifos	0.50 (0.32, 0.78)	0.43 (0.29, 0.63)	0.43 (0.30, 0.62)	0.25 (0.17, 0.37)
Lorsban®	0.73 (0.49, 1.10)	0.60 (0.40, 0.90)	0.46 (0.30, 0.71)	0.33 (0.26, 0.42)
permethrin	> 0.2	> 0.2	> 0.2	> 0.2
Mosq.-B-Gone®	> 0.2	> 0.2	> 0.2	> 0.2

Chlorpyrifos LC50 comparison

Species	Duration	LC50 (μg/l)
L. siliquoidea		
Glochidia	48 h	430
Juvenile	96 h	250
Roach	96 h	250
Rainbow trout	96 h	3
D. magna	48 h	1.7

Results summary: Phase II

- Technical grade and formulation chlorpyrifos were acutely toxic to glochidia and juveniles
- Roundup was substantially more toxic than technical grade glyphosate for glochidia & juveniles
- Toxicity of formulation atrazine, chlorpyrifos, and permethrin similar to technical grade
- Chronic toxicity of formulations to juveniles forthcoming

Conclusions

- Some pesticides and formulations are acutely toxic to glochidia & juveniles
- Some pesticide formulations may be more toxic than technical grade → implications
- Glochidia and juveniles have similar sensitivity to pesticides
- Chronic effects of atrazine on survival & growth indicate further investigation warranted
- Toxicity of pesticides to adults?

Summer/Fall 2005

- Chronic juvenile tests (21 d)
  - Survival
  - Growth
- Adult tests
  - Lifestage sensitivity comparisons
    - Chlorpyrifos, NaCl
  - Genotoxicity
  - Steroid hormones

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